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Molecular Docking Study of Naturally Derived β-mangostin with Antiapoptotic Bcl-2 Proteins Toward Oral Cancer Treatment

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ABSTRACT

Targeting the apoptosis-inducing pathway has drawn much attention in searching for a novel anticancer drug. Bcl-2 is the most studied anti-apoptotic protein, recognised in aiding in cell survival and overexpressed in most cancer cells resulting in cancer resistance toward conventional treatment. The inhibition of Bcl-2 proteins become the main target for inducing apoptosis in cancer cells. β-mangostin received minimum attention in investigating anticancer properties as compared to its family such α-mangostin. We performed molecular docking of β-mangostin, doxorubicin (in silico control) and ABT-737 (co-crystal Bcl-2 inhibitor) against antiapoptotic Bcl-2 protein using PyMol, Discovery Studio Biovia 2021, AutoDock Vina, and AutoDock Tools version 1.5.7. The result demonstrates for the first time that β-mangostin showed an optimum binding affinity with Bcl-2 (ΔG −7.3 kcal/mol), similar to those shown by doxorubicin. The present results indicate that β-mangostin could potentially serve as Bcl-2 protein inhibitors, which would consequently lead to an apoptotic process in oral cancers. The present data warrant validation using in vitro and in vivo assays.

Keywords: *Bcl-2, β-mangostin; apoptosis, binding affinity, in silico molecular docking*

1. INTRODUCTION

Oral cancer is a serious health threat, considered the leading cause of death from oral illness across nations. The WHO revealed the incidence of oral cancer cases has increased to 377,713 cases with 177,757 oral cancer fatalities in 2020 [1,2]. Currently, the 5-year survival rates across the region remain lower than 50 percent and Malaysian patients with oral cancer had a survival rate of 40.9 percent, which is lower than the global average [3,4,5]. The patient commonly suffered a recurrence and progressive disease due to chemotherapy resistance developed

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during/after treatment. The chemotherapy regimens which are produced synthetically demonstrated non-selective cytotoxicity against cancer and healthy cells [6]. Conversely, recent studies recommend naturally derived bioactive compounds to be explored further since they have high selective toxicity toward cancer cells despite having negligible toxicity on normal cells [7]. This could be an alternative chemotherapeutic drug regimen for oral cancer treatment.

Xanthone is an interesting natural phytochemical that possess a wide range of pharmacological activities, including anti-bacterial, anti-virus, anti-allergy, anti-inflammatory, anti-malarial, anthelmintics, antiprotozoa, antioxidant, hepatoprotective and anticancer activities [8,9]. Over the past few years, the anticancer properties of xanthones have been extensively investigated. α-mangostin and β-mangostin are xanthones that were successfully isolated from mangosteen fruit. Numerous studies reported the α-mangostin xanthone have exerted anticancer properties [8,10,11]. However, anticancer properties of β-mangostin ($C_{25}H_{28}O_6$) that co-exist with α-mangostin have received minimal interest [8,12,13].

Interestingly, a previous study reported that β-mangostin isolated from *G. malaccensis* showed stronger cytotoxicity than α-mangostin on cancer cells tested [14]. In the year 2017, studies have revealed that β-mangostin has a cytotoxic effect against murine leukemia (WEHI-3) cells and HL60 cell line in vitro [13,15]. Moreover, β-mangostin could also inhibit the growth of MCF-7, a breast cancer cell line [16]. Nevertheless, the anticancer properties of β-mangostin for the treatment and/or prevention of oral cancer have not been explored yet.

Apoptosis is a natural cell death mechanism that plays a critical role in cell growth and tissue development [17]. It is called the clean type of cell death in which the chromatin condenses, the DNA fragmented, apoptotic vesicles formed and then quickly phagocytised by macrophages, resulting in the cell's disappearance without any inflammatory [18]. Thus, inducing the apoptosis pathway is considered the primary goal in treating cancer cells.

The B-cell lymphoma (Bcl-2) family protein consists of proapoptotic and antiapoptotic proteins, which play a crucial role in regulating the intrinsic apoptosis pathway [19]. Bcl-2 is the most studied anti-apoptotic protein, recognised to preserve mitochondrial integrity and aid in cell survival [20]. This anti-apoptotic protein is overexpressed in most cancer cells as compared to pro-apoptotic protein resulting in cancer resistance toward conventional treatment [21]. The inhibition of Bcl-2 proteins become a critical target for inducing apoptosis in cancer cells. Thus, phytochemical compounds that could inhibit Bcl-2 protein function have received much attention in recent years as a novel anticancer candidate.

In silico molecular docking has become an important tool for the exploration and development of various novel drugs. This approach predicts how a protein (enzyme) interacts with small molecules (ligands), which analyse the ligand-protein binding pose (conformation and orientation) and binding free energy (binding affinity) [22]. The establishment of a virtual ligand-protein interactions model at the atomic level later could be confirmed using *in vitro* and *in vivo* approaches thus reducing time and cost in drug discovery [23]. Herein, this study aimed to investigate the binding interaction between naturally-derived β-mangostin against potential antiapoptotic Bcl-2 protein using molecular docking software in terms of, the binding affinities and the presence of non-covalent interactions.

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2. MATERIALS AND METHOD

2.1 Software and program

PyMol and *Discovery Studio Biovia 2021* were used to visualise and modify the receptor and ligand structures. *AutoDock Vina* was the main docking program used in this work. The .PDBQT file format preparation and the grid box determination were done using *AutoDock Tools version 1.5.7*. Post docking analysis was done using *PyMol* and *DS Biovia 2021*.

2.2 Preparation of ligand structures for β-mangostin, doxorubicin and ABT-737

The 3D structure of β-mangostin and doxorubicin (*in silico* control) were downloaded in the Spatial Data File (.SDF) file format from PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/). The .SDF file format was converted into the Protein Bata Bank Files (.PDB) format using *Discovery Studio Biovia 2021*. *AutoDock Tools 1.5.7 (ADT)* was then used to prepare ligand structure with Gasteiger changes and rotatable bonds. Structures in the .PDB file format was then converted to the Protein Data Bank, Partial Charge & Atom Type (PDBQT) file format using ADT and made it eligible for molecular docking using the *AutoDockVina* [23].

2.3 Preparation of macromolecule structures of the protein Bcl-2

The crystal structure of the Bcl-2 protein (PDB: 6QGG) was downloaded from the Research Collaboratory for Structural Bioinformatics website (http://www.rcsb.org). Protein inhibitors were separated using *Discovery Studio Biovia 2021* and used in the redocking step. *ADT* software was used to prepare the required files for *AutoDock Vina* by removing water, adding polar hydrogen, computing Gasteiger charges to protein structures, and converting protein structures from the .PDB file format to .PDBQT file format [24-26].

2.4 Grid Box Determination

The location of the grid box was selected based on the known original inhibitor's location using *ADT* [23]. The grid coordinates were confirmed after serial redocking steps of the inhibitor to the protein with a root-mean-square deviation (RMSD) value below 2 Ångström (Å) which represent good reproduction of the correct pose of predicted structural conformation as compared to observed structural conformation from actual experiments. Meanwhile, the size of the grid dimension was determined based on the sizes of each ligand.

2.5 Molecular Docking

Molecular docking was performed using the *AutoDock Vina* program. The configuration file was engaged by opening notepad to run *AutoDock Vina. ADT* was required to prepare the output.PDBQT file for ligand and to set the size and the centre of the grid box. The grid size dimension and the grid centre were set at 14 × 24 × 12 (x, y, and z) points, and the grid centre was designated at x, y, and z dimensions of -15.690, 14.488, and -9.525, respectively, with a grid spacing of 1.000 Å. The prepared file was saved in .PDBQT file format. Ligand-binding affinities were predicted as negative Gibbs free energy (ΔG) scores (kcal/mol), which were calculated based on the *AutoDock Vina* scoring function [27]. The predicted binding affinity showed how strong a ligand binding to the receptor. Post-docking analyses were visualised

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using *PyMOL* and *Discovery Studio Biovia 2021*, which showed the sizes and locations of binding sites, hydrogen-bond interactions, hydrophobic interactions, and bonding distances. Binding poses of each ligand were observed and their interactions with the protein were characterised, and the best and most energetically favourable conformations of each ligand were selected.

3. RESULT

The docking protocol was validated by redocking ABT-737 to Bcl-2 [PDB: 6QGG]. Figure 1 showed the result of redocking ABT-737 inhibitor (blue) and ABT-737 crystallographic conformation (green). The superimposition fits between the ABT-737 inhibitor (green) and the ABT-737 crystal conformation (blue) which describes the position of the binding site between the ligand and the receptor. The RMSD value is 1.321 Å. RMSD has often been used to measure the quality of reproduction of a known crystallographic binding pose by a computational method. The lower the RMSD represents good reproduction of the correct binding pose.

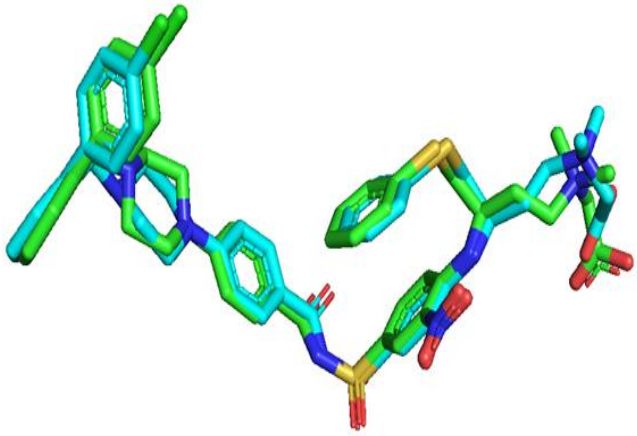

Figure 1: Redocking ABT-737 to the binding site. Crystallised conformation is shown in green colour. The best redocked pose of ABT-737 is shown in blue colour.

The lowest binding energy was -10.7 kcal/mol showed by ABT-737. β-mangostin has an equally strong affinity with doxorubicin, each showing a binding affinity -7.3 kcal/mol against Bcl-2 (Table 1). These results indicate that, of the three ligands, ABT-737 possesses the highest binding affinity for Bcl-2.

Table 1: Binding affinity value between β-mangostin and control ligand at the binding site of Bcl-2 receptor

Ligand	Binding energy (binding affinity) generated between the ligand and the Bcl-2 receptor in each conformation, ΔG (kcal/mol)								
	1	2	3	4	5	6	7	8	9
β-mangostin	-7.3	-7.2	-7.2	-7.1	-6.9	-6.9	-6.8	6.4	-6.3
Doxorubicin	-7.3	-6.9	-6.8	-6.8	-6.7	-6.7	-6.5	-6.5	-6.2
ABT-737	-10.7	-10.3	-9.5	-8.3	-7.9	-7.9	-	-	-

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During analyses, we recorded Bcl-2 amino acid residues that are involved in a hydrogen bond, hydrophobic, and electrostatic interactions with the ligands using *AutoDock Vina* (Figure 2, Figure 3, Figure 4 and Table 2).

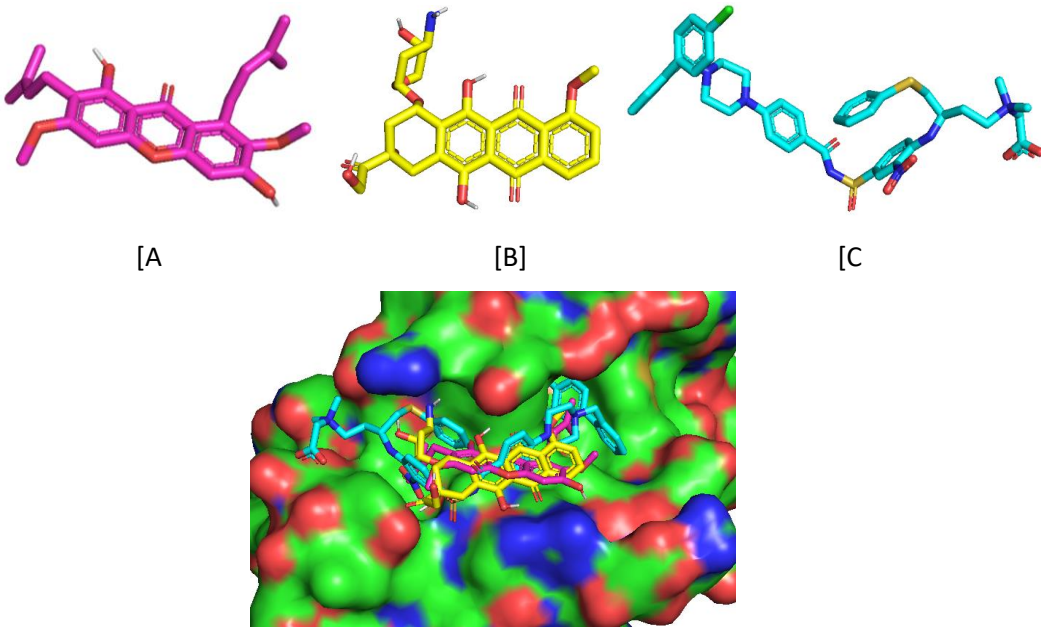

Figure 2: Binding conformation of the compounds in the binding site. (A) β-mangostin is shown in magenta carbons. (B) Doxorubicin is shown in yellow carbons. (C) Compound ABT-737 is shown in cyan carbons. (D) Superimposition of β-mangostin, Doxorubicin and redocked of ABT-737 inhibitor.

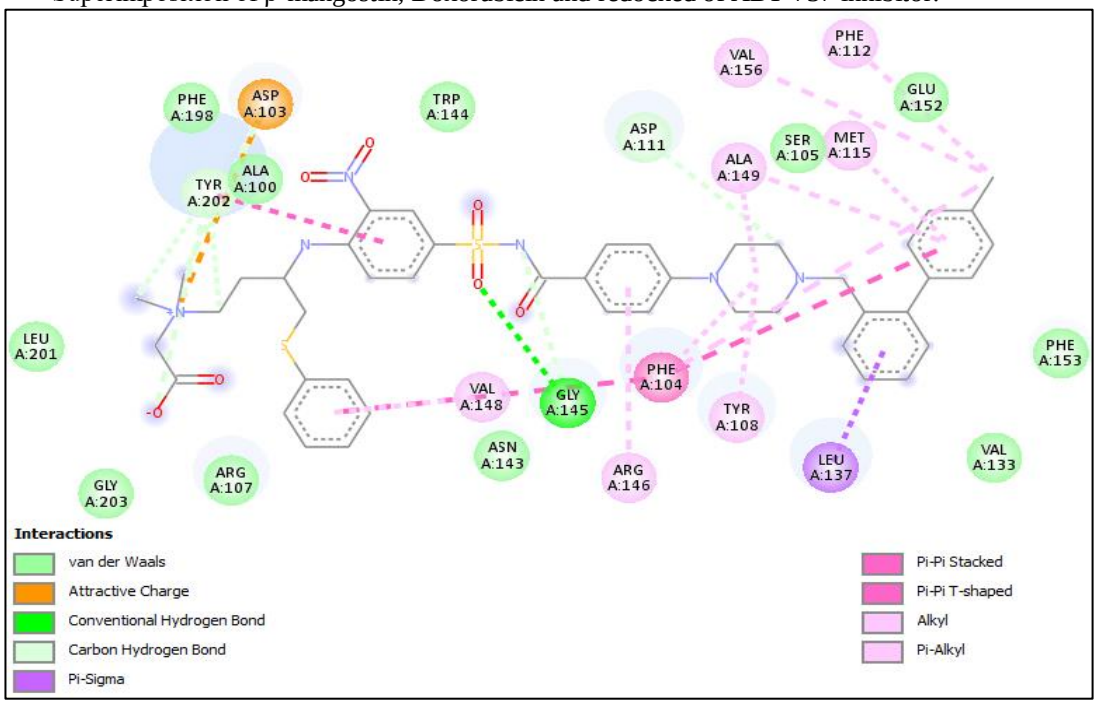

Figure 3: 2D illustration of docked ABT-737-Bcl-2 complex interaction was visualised by Discovery Studio Biovia 2021.

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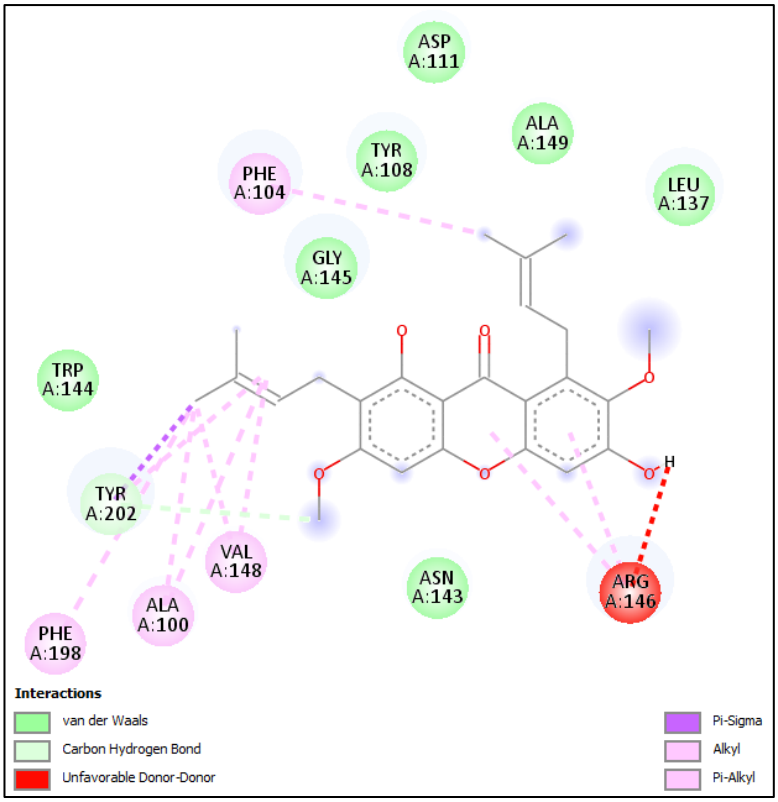

Figure 4: 2D illustration of docked β-mangostin-Bcl-2 complex interaction was visualised by Discovery Studio Biovia 2021.

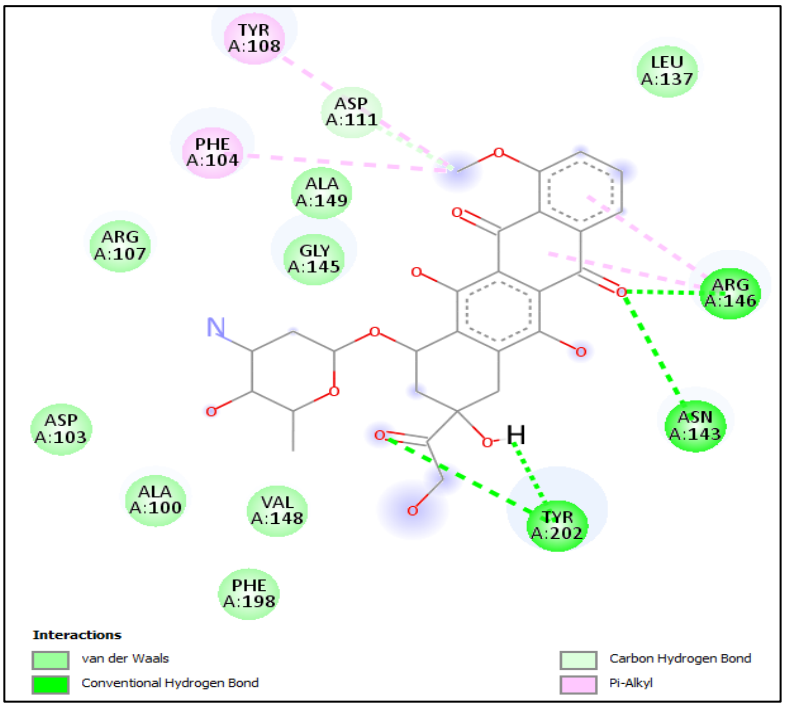

Figure 5: 2D illustration of docked Doxorubicin-Bcl-2 complex interaction were visualised by Discovery Studio Biovia 2021.

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Table 2: Amino acid residues involved in the interaction between the ligands and the BCL-2 receptor on hydrogen bonding, hydrophobic interactions and electrostatic interactions, respectively.

Ligand	Binding affinity, ΔG (kcal/mol)	Amino acids involved and distance (Å)		
		Hydrogen-Binding Interaction	Hydrophobic Interaction	Electrostatic Interaction
β-mangostin	-7.3	Tyr202 (3.64)	Tyr202 (3.96), Ala100 (5.12), Ala100 (4.01), Val148 (4.97), Val148 (4.24), Phe104 (5.01), Phe198 (4.96), Tyr202 (4.92), Tyr202 (5.13), Arg146 (5.28), Arg146 (4.50)	-
Doxorubicin	-7.3	Asn143 (3.14), Arg146 (3.10), Tyr202 (2.90), Tyr202 (2.03), Asp111 (3.68)	Phe104 (4.93), Tyr108 (4.70), Arg146 (4.82), Arg146 (4.69)	-
ABT737	-10.7	Gly145 (3.16), Gly145 (3.42), Tyr202 (3.63), Asp111 (3.20), Tyr202 (3.53), Tyr202 (3.54), Asp103 (3.65)	Leu137 (3.83), Tyr202 (3.94), Phe104 (5.17), Phe104 (5.13), Ala149 (4.68), Val156 (5.03), Phe104 (5.05), Phe104 (5.07), Tyr108 (5.37), Phe112 (4.64), Met115 (4.60), Ala149 (4.96), Arg146 (5.47), Val148 (5.16)	Asp103 (4.48)

The interaction between β-mangostin and Bcl-2 formed a hydrogen bond involving the amino acid residue Tyr202 with a distance of 3.64Å. Eleven hydrophobic interactions also occur involving the amino acids Tyr202 (3.96 Å), Ala100 (5.12 Å), Ala100 (4.01 Å), Val148 (4.97 Å), Val148 (4.24 Å), Phe104 (5.01 Å), Phe198 (4.96 Å), Tyr202 (4.92 Å), Tyr202 (5.13 Å), Arg146 (5.28 Å) and Arg146 (4.50 Å). There was no electrostatic interaction between β-mangostin compound and Bcl-2.

Doxorubicin compounds formed five hydrogen bonds with Bcl-2, involving amino acids Asn143 (3.14 Å), Arg146 (3.10 Å), Tyr202 (2.90 Å), Tyr202 (2.03 Å) and Asp111 (3.68 Å). Meanwhile, four hydrophobic interactions occurred involving amino acids Phe104 (4.93 Å), Tyr108 (4.70 Å), Arg146 (4.82 Å) and Arg146 (4.69 Å). The interaction between doxorubicin and Bcl-2 did not result in an electrostatic interaction.

ABT-737 redocking interaction with Bcl-2 were supported by three hydrogen bonds at residues Gly145 (3.16 Å), Gly145 (3.42 Å), Tyr202 (3.63 Å), Asp111 (3.20 Å), Tyr202 (3.53 Å), Tyr202 (3.54 Å) and Asp103 (3.65 Å); and by fourteen hydrophobic interactions at residues Leu137 (3.83 Å), Tyr202 (3.94 Å), Phe104 (5.17 Å), Phe104 (5.13 Å), Ala149 (4.68 Å), Val156 (5.03 Å), Phe104 (5.05 Å), Phe104 (5.07 Å), Tyr108 (5.37 Å), Phe112 (4.64 Å), Met115 (4.60 Å), Ala149 (4.96 Å), Arg146 (5.47 Å) and Val148 (5.16 Å). An electrostatic interaction was also identified with Asp103 (4.48 Å).

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4. DISCUSSION

Molecular docking is becoming a popular approach for new drug exploration and development. It is a powerful tool in analysing the structure-activity relationship [19]. This approach enables us to predict binding conformation and binding energy of small molecules ligand against targeted protein subsequently facilitate the determination of potential ligand for pharmaceutical use [24]. In addition, molecular docking saves time and cost as compared to traditional laboratory experiments.

The antiapoptotic protein exploited in this study is Bcl-2, which play an important role in regulating the intrinsic apoptotic signalling pathway. Bcl-2 protein has previously been examined in the molecular docking aspect for inducing apoptosis in cancer cells using small molecules like phenothiazine and piperine [28,29,30]. In this study, protein-ligand docking was applied to investigate the potential interaction of β-mangostin in inhibiting anti-apoptotic Bcl-2 protein.

Our results show that all ligands (β-mangostin, doxorubicin and ABT-737) appear to have good docking interaction with Bcl-2 protein (Table 1). The binding affinity that occurs by the β-mangostin is equal to doxorubicin which is ΔG value of -7.3 kcal/mol. Meanwhile, the binding affinity produced by ABT-737 inhibitors redocking was -10.7 kcal/mol. Previously, the ABT-737 compound was identified to have interaction with Bcl-2 protein experimentally [31]. The value binding affinity produced by ABT-737 inhibitors redocking in this study is similar to recent docking studies by Afriza (2018) [24] which indicated the docking protocols are acceptable. The ligand-receptor interaction tends to be at the lowest energy state, thus the ligand will bind spontaneously. So, the lesser value of ΔG, the more stable the ligand-receptor interaction will be. Hence, negative ΔG scores indicate the stability of the resulting complexes with receptor molecules, and this is an essential characteristic of efficacious drugs [23].

The presence of hydrogen-binding interaction, hydrophobic interaction and electrostatic interaction in the ligand-protein docking with Bcl-2 protein was observed to contribute to the different binding affinities values (Table 2). Even though binding affinities are helpful in ligand docking to the protein's active pocket, non-covalent molecular interactions (hydrogen bonds, hydrophobic interactions, and electrostatic interactions) with essential amino acid residues are indicative of ligand docking in favourable conformations [23]. Hydrophobic interactions are the most essential contributors to protein stability. Hydrogen bond also helps to keep proteins stable, but to a lesser extent than hydrophobic interactions, even in the tiniest globular proteins [32]. Accordingly, hydrophobic interaction is the primary determinant of folding configuration equilibrium in numerous native proteins [32]. Electrostatic interactions also influence the protein binding affinity, structure, chemical properties, stability and biological reactivity [24].

In this study, redocking of ABT-737 possess seven hydrogen bonds, fourteen hydrophobic and one electrostatic interaction. Interestingly, β-mangostin and doxorubicin shared the same binding affinity value, with doxorubicin's interaction appearing to be balanced between hydrogen bond and hydrophobic interaction, while β-mangostin having excessive hydrophobic bonds than hydrogen bonds (Table 2). This implies that all types of interactions contributed together to forming binding affinity values. Apparently, these three ligands interact on the same residue on the Bcl-2 receptor for hydrogen bond (Tyr202) and hydrophobic interaction (Phe104 and Arg146). A previous study demonstrated that Tyr202 located at the Bcl-2 region is also

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responsible for forming a hydrogen bond with many Bcl-2 protein inhibitors [33]. Nevertheless, β-mangostin still has a good binding affinity so that it can potentially be an inhibitor for Bcl-2 activity.

5. CONCLUSION

Our findings conclude that β-mangostin is possibly able to act as potential inhibitors for the targeted Bcl-2 proteins as supported by the high binding affinities and various binding interactions which subsequently induce apoptotic signalling pathways in oral cancer cells. However, further in vitro and in vivo experiments are required to validate these in silico results.

6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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